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A dual role for interferon γ signalling in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a malignant disease with heterogeneous aetiology. Despite this heterogeneity, HCCs have been classified into distinct subtypes based on candidate approaches or global screening methods [1–6]. Moreover, whole genome sequencing of HCCs and putative secondary growths have defined genetic changes associated with intrahepatic metastasis [7]. However, the identification of driver mutations in HCC is still a challenging task [8]. Mouse models are valuable tools to identify the contribution of defined genetic changes to HCC formation. As recently reviewed by Gen-Sheng Feng, controversial results have been obtained in mice for several candidate genes that seemed to display both, tumour-promoting and tumour-suppressive activities [9]. These controversial findings are reconciled by the fact that gene activities can influence HCC formation by two mechanisms: (1) a candidate gene is required for tumour cell proliferation or survival (oncogenic function); (2) a candidate gene is essential for survival of hepatocytes, thereby preventing chronic liver injury and regeneration (anti-oncogenic function). These mechanisms might operate in all tissues with high regenerative capacity. Consequently, chronic liver injury induced by ablation of an essential candidate gene could drive formation of HCCs with specific mutations that override tumour cell-autonomous requirements for the candidate gene. This dual mechanism exacerbates our interpretations of cell-autonomous gene functions in HCC formation when corresponding mouse models differ from control groups with respect to chronic liver injury. Moreover, many models do not reflect the human aetiology of HCC, which is associated with inflammation, chronic liver damage, and liver fibrosis.

In this issue of the *Journal of Hepatology*, Meng *et al.* employed mice lacking the farnesoid X receptor ($FXR^{-/-}$) to investigate oncogenic functions of type II interferon (IFNγ) signalling in HCC formation [10]. $FXR^{-/-}$ mice represent a model of HCC induced by metabolic dysfunction and the authors have recently demonstrated that this model can recapitulate several aspects of human HCC [11]. They showed that FXR downregulation in human HCCs was mediated by cytokines that interfered with HNF1α (hepatocyte nuclear factor 1α) chromatin binding and proposed multiple mechanisms on how loss of FXR could support hepatocarcinogenesis [11]. FXR is required for hepatocyte survival thereby preventing chronic liver injury but also regulates expression of target genes, such as SHP (small heterodimer partner), that are implicated in hepatocarcinogenesis. Moreover, chronic liver injury in $FXR^{-/-}$ mice led to infiltration of macrophages and elevated hepatic expression of inflammatory cytokines which induced abnormal hepatocyte proliferation, a driving force for expansion of transformed cells [11]. One of the most prominently upregulated cytokines in $FXR^{-/-}$ mice was IFNγ [12]. This cytokine, however, suppresses formation of various tumour types by cell-autonomous and non-cell-autonomous mechanisms. IFNγ stimulates immune surveillance functions of the innate immune system and can induce expression of immunomodulatory factors such as MHC (major histocompatibility complex) molecules in tumour cells. Moreover, IFNγ signalling interferes with tumour cell proliferation and promotes apoptosis. Many of these functions are mediated by the transcription factor STAT1 (signal transducer and activator of transcription 1). Consistent with the anti-proliferative and pro-apoptotic activities of IFNγ/STAT1 signals, tumour cells try to escape from this pathway and acquire resistance by different means (downregulation of IFNγ receptors; deletion of the genomic IFNγ locus; promoter methylation of STAT1 or other factors implicated in IFNγ signalling which makes tumour cells refractory to IFNγ signals) [13]. The tumour-suppressive activity of IFNγ/STAT1 is partly due to mutually interfering interactions between STAT1 and the closely related transcription factor STAT3 (signal transducer and activator of transcription 3). In contrast to STAT1, STAT3 is considered to promote formation of many tumours including HCC. However, administration of the carcinogen DEN (diethylnitrosamine) in mice with conditional inactivation of STAT3 in the liver has provided controversial results with two studies suggesting an oncogenic activity [14,15] and one study suggesting an anti-oncogenic activity of STAT3 [16]. We

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Abbreviations: T, T cell; NK, natural killer cell; NKT, natural killer T cell; SC, stellate cell; KC, Kupffer cell; TNFα, tumour necrosis factor alpha; TGFβ, transforming growth factor beta; TRAIL, TNF-related apoptosis-inducing ligand; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; gp130, glycoprotein gp130; IFNGR1, 2, interferon gamma receptor 1, 2; JAK1, 2, Janus kinase 1, 2; ROS, reactive oxygen species; Mdm2, murine double minute 2; IFITM1, interferon-induced transmembrane protein 1; IRF1, interferon regulatory factor 1; iNos, inducible nitric oxide synthase; NFκB, nuclear factor kappa B.

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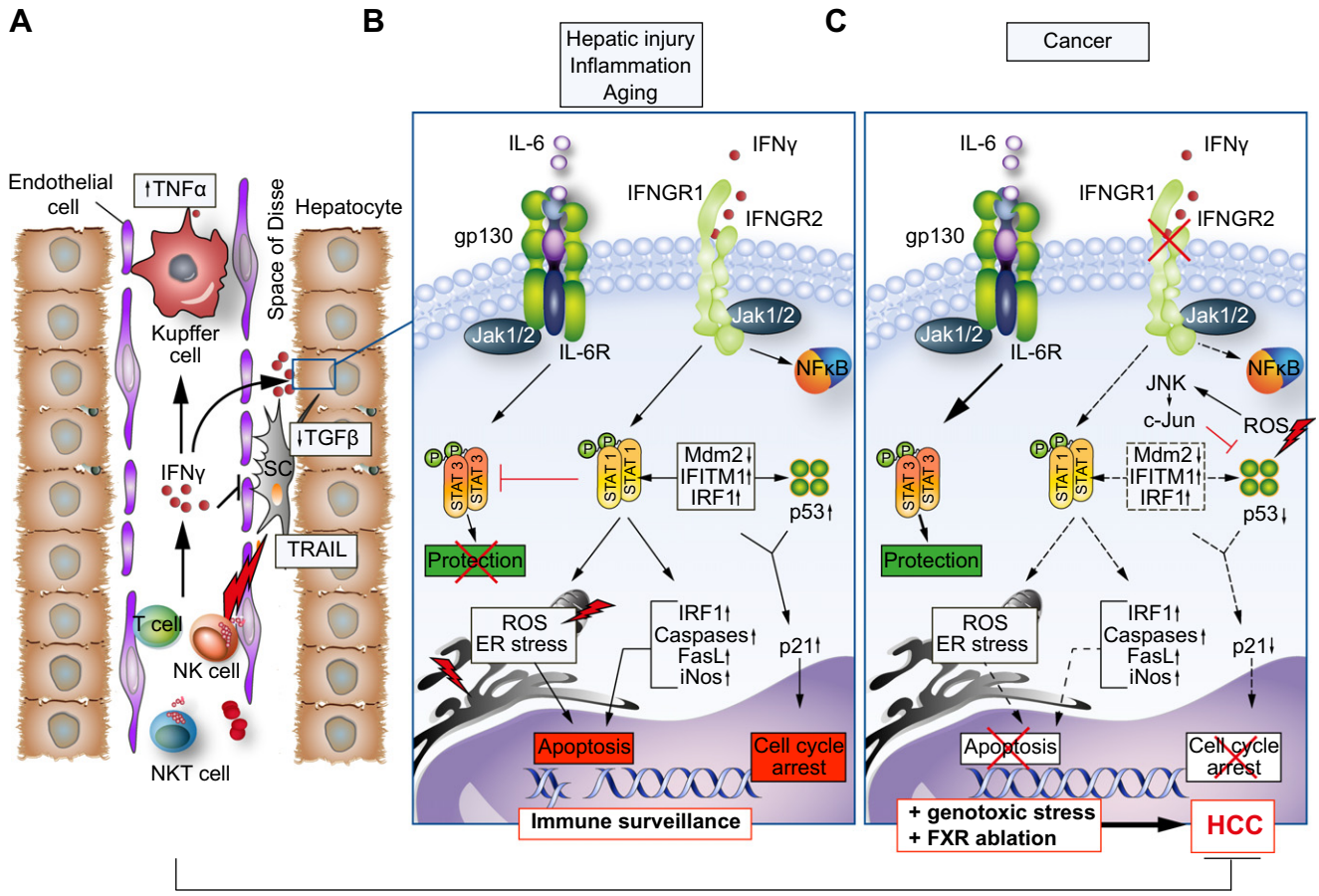

Fig. 1. Functions of IFNγ signalling in hepatocellular carcinoma. (A) The major sources of IFNγ in the liver are sinusoidal NK, NKT and T cells, attracted during inflammation and liver injury triggered by different insults. IFNγ acts on Kupffer cells and macrophages and promotes production of cytokines such as TNFα. Moreover, NK cells kill stellate cells and IFNγ interferes with TGFβ signalling in stellate cells thereby executing antifibrogenic functions. (B) IFNγ signalling in hepatocytes is amplified during hepatic injury, inflammation and ageing via upregulation of IFNγ receptor or IFNγ ligand expression. IFNγ/STAT1 interferes with hepatoprotective and tumour-promoting activities of STAT3, induces apoptosis of hepatocytes (in synergy with TNFα) via several mechanisms (p53-independent) and promotes cell cycle arrest in an IRF1/p53/p21-dependent manner. STAT1 and p53 directly interact with each other and co-regulate expression of p21. Moreover, STAT1 can increase p53 protein levels via repression of Mdm2 expression and other molecular mechanisms. The pro-apoptotic activity of STAT1 is modulated by NFκB, a hepatoprotective transcription factor that is also activated by IFNγ. These molecular mechanisms integrate anti-oncogenic activities of IFNγ/STAT1. (C) Upon hepatocyte transformation, IFNγ signalling is compromised and IL-6/STAT3 signalling is enhanced leading to STAT3 activation and reduced STAT1 activity. The latter results in release from cell cycle arrest and reduced IFNγ/STAT1-induced apoptosis. Moreover, STAT1-dependent mechanisms for stabilization of p53 are ablated. These molecular events might contribute to hepatocyte transformation in a tumour cell-autonomous manner. Moreover, IFNγ signalling prevents accumulation of ROS (partially via NFκB) and activation of JNK thereby executing hepatoprotective functions. Consequently, loss of IFNγ leads to necrotic liver damage, a condition that promotes HCC formation especially when additional insults such as genotoxic or metabolic stress (FXR ablation) are present.

have performed a similar experiment and found significant reduction of DEN-induced HCC formation in the absence of STAT3 (unpublished) suggesting an oncogenic function. The mutual interaction between STAT1 and STAT3 became particularly apparent when STAT1 or STAT3 activities were selectively ablated. Deletion of STAT1 switched the pro-apoptotic and anti-proliferative activities of IFNs to survival and proliferation signals in various cell types. This functional switch was at least in part due to aberrant activation of STAT3 by IFN signals that occurred only in the absence of STAT1. In contrast, STAT1 was activated by glycoprotein gp130-inducing signals when STAT3 was ablated. Consequently, STAT3-deficient fibroblasts responded to IL-6 (interleukin-6) with substantial STAT1 activation that resulted in unusual IL-6-mediated activation of IFN responsive genes [17]. The molecular mechanisms that underlie the reciprocal influence of STAT1 and STAT3 activation include competition for common cytokine receptors, the implication of SOCS (suppressor of cytokine) proteins and the mutual inactivation of STATs by sequestration into STAT1:STAT3 heterodimer complexes [18].

The report by Meng *et al.* shed new light on the role of IFNγ and the mutual interplay of STAT transcription factors in hepatoprotection and HCC formation [10]. Abolishing IFNγ signalling led to increased hepatic damage and tumour load in livers of $IFN\gamma^{-/-}$, $IFN\gamma^{-/-}$ $FXR^{-/-}$ double-deficient and $IFN\gamma^{-/-}$ mice treated with DEN [10]. These experiments provide new insights into tumour-suppressive activities of IFNγ signals that seem to operate not only in tumour cells but also through hepatoprotective mechanisms (Fig. 1). The hepatoprotective activity of IFNγ was age-dependent since old $IFN\gamma^{-/-}$ mice displayed hepatic inflammation and elevated levels of liver damage parameters that were not obvious in young mice. Interestingly, levels of IFNγ increased in humans and mice during ageing indicating that an age-dependent physiologic mechanism co-ordinately regulates IFNγ levels and IFNγ responsiveness of hepatocytes.

Editorial

Increased tumour load in double-deficient *IFNγ*$^{-/-}$ *FXR*$^{-/-}$ mice and *IFNγ*$^{-/-}$ mice treated with DEN might result from aggravated chronic liver damage and regeneration. Moreover, aggravated liver damage was accompanied by several molecular changes that promote HCC formation including STAT3 activation, reduction of NFκB activity, decreased p53 expression and elevated activation of JNK (Jun aminoterminal kinase) [10]. STAT3 activation might be due to the aforementioned mutual interaction between IFNγ/STAT1 and IL-6/STAT3 signalling pathways, which was further demonstrated *in vitro*. Activation of JNK, mediated by reactive oxygen species (ROS) and blunted NFκB activation, could contribute to p53 downregulation via its target protein c-Jun, a transcription factor known to repress expression of p53 in hepatocarcinogenesis [19]. In this regard, JNK could act synergistically with known IFNγ/STAT1-dependent mechanisms for p53 regulation (Fig. 1). However, these mechanisms seemed to operate predominantly during HCC initiation because p53 expression was not affected in established tumours of *IFNγ*$^{-/-}$ *FXR*$^{-/-}$ mice. Recently, Katz *et al.* have investigated p53 functions in HCC. They demonstrated formation of HCCs with stem cell-like characteristics (Alb^{+} and K19^{+}) in aged mice with conditional ablation of p53 in hepatocytes [20]. It would be interesting to investigate whether the short window of p53 loss during tumour initiation is sufficient to promote formation of bilineal HCCs with stem cell-like characteristics in *IFNγ*$^{-/-}$ *FXR*$^{-/-}$ mice.

In summary, the study by Meng *et al.* has proposed an important IFNγ-dependent cellular and molecular network between innate immune cells and hepatocytes. This network could provide protection from liver damage and HCC formation during viral hepatitis or autoimmune hepatitis that are associated with attraction of several hematopoietic producers of IFNγ. Paradoxically, gain and loss of IFNγ signalling lead to hepatocyte death but only loss of IFNγ promotes HCC formation. It remains to be investigated how the concerted action with additional IFNγ responses such as cell cycle arrest modulates anti-oncogenic activities of IFNγ-induced apoptosis.

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Calvisi DF, Ladu S, Gorden A, Farina M, Lee JS, Conner EA, et al. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. J Clin Invest 2007;117:2713–2722.

[2] Hou J, Lin L, Zhou W, Wang Z, Ding G, Dong Q, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. Cancer Cell 2011;19:232–243.

[3] Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001;120:1763–1773.

[4] Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology 2004;40:667–676.

[5] Roessler S, Long EL, Budhu A, Chen Y, Zhao X, Ji J, et al. Integrative genomic identification of genes on 8p associated with hepatocellular carcinoma progression and patient survival. Gastroenterology 2012;142:957–966, e912.

[6] Shen J, Wang S, Zhang YJ, Kappil M, Wu HC, Kibriya MG, et al. Genome-wide DNA methylation profiles in hepatocellular carcinoma. Hepatology 2012;55:1799–1808.

[7] Tao Y, Ruan J, Yeh SH, Lu X, Wang Y, Zhai W, et al. Rapid growth of a hepatocellular carcinoma and the driving mutations revealed by cell-population genetic analysis of whole-genome data. Proc Natl Acad Sci USA 2011;108:12042–12047.

[8] Sawey ET, Chanrion M, Cai C, Wu G, Zhang J, Zender L, et al. Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by oncogenomic screening. Cancer Cell 2011;19:347–358.

[9] Feng GS. Conflicting roles of molecules in hepatocarcinogenesis: paradigm or paradox. Cancer Cell 2012;21:150–154.

[10] Meng Z, Wang X, Gan Y, Zhang Y, Zhou H, Van Ness C, et al. Deletion of IFNγ enhances hepatocarcinogenesis in FXR knockout mice. J Hepatol 2012;57:1004–1012.

[11] Liu N, Meng Z, Lou G, Zhou W, Wang X, Zhang Y, et al. Hepatocarcinogenesis in FXR−/− mice mimics human HCC progression that operates through HNF1alpha regulation of FXR expression. Mol Endocrinol 2011;26:775–785.

[12] Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. Cancer Res 2007;67:863–867.

[13] Horras CJ, Lamb CL, Mitchell KA. Regulation of hepatocyte fate by interferon-gamma. Cytokine Growth Factor Rev 2011;22:35–43.

[14] He G, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, et al. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. Cancer Cell 2010;17:286–297.

[15] Wang H, Lafdil F, Wang L, Park O, Yin S, Niu J, et al. Hepatoprotective versus oncogenic functions of STAT3 in liver tumorigenesis. Am J Pathol 2011;179:714–724.

[16] Bard-Chapeau EA, Li S, Ding J, Zhang SS, Zhu HH, Princen F, et al. Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. Cancer Cell 2011;19:629–639.

[17] Mair M, Blaas L, Osterreicher CH, Casanova E, Eferl R. JAK–STAT signaling in hepatic fibrosis. Front Biosci 2011;17:2794–2811.

[18] Murray PJ. The JAK–STAT signaling pathway: input and output integration. J Immunol 2007;178:2623–2629.

[19] Eferl R, Ricci R, Kenner L, Zenz R, David JP, Rath M, et al. Liver tumor development. c-Jun antagonizes the proapoptotic activity of p53. Cell 2003;112:181–192.

[20] Katz SF, Lechel A, Obenauf AC, Begus-Nahrmann Y, Kraus JM, Hoffmann EM, et al. Disruption of Trp53 in livers of mice induces formation of carcinomas with bilineal differentiation. Gastroenterology 2012;142:1229–1239, e1223.